Defective T cell immunity in HBV: why therapeutic vaccination needs a helping hand

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Word Count: 5001

Abstract

Hepatitis B virus (HBV) remains a huge cause of morbidity and mortality worldwide; treatments that can induce a state of 'functional cure' in patients chronically infected with this hepatotropic non-cytopathic virus are desperately needed. Attempts to use therapeutic vaccines to expand the weak antiviral T cell response and induce sustained immunity have not been successful to date. However, there has been exciting progress in defining the molecular defects that need to be overcome in order to harness T cell immunity. We now have a large arsenal of immunotherapeutic agents as well as direct-acting antivirals targeting multiple steps of the viral lifecycle emerging on the horizon. Here we discuss how to translate new insights into T cell manipulation, combined with better understanding of patient heterogeneity, in order to optimise therapeutic vaccines. We review the opportunities and risks of boosting endogenous T cell responses by the next generation of therapeutic vaccine/immunotherapy combinations.

Introduction: Moving towards a functional cure of HBV

Recent studies have highlighted the huge and increasing burden of disease attributable to HBV infection. This small, hepatotropic DNA virus is thought to have infected one third of the world's population, with an estimated 260 million people currently persistently infected. HBV is non-cytopathic; it can replicate at extremely high levels without causing any liver damage or conversely, can trigger immunopathology leading to liver cirrhosis and hepatocellular carcinoma (HCC). These complications are responsible for around 780,000 deaths a year, making HBV one of the most common causes of death worldwide(1).

The current mainstays of treatment for HBV are a course of pegylated interferon-alpha (PEG-IFNα) or suppressive treatment with the nucleos(t)ide analogues Tenofovir or Entacavir. The lack of sustained off-treatment responses necessitates long-term therapy, with its attendant potential problems of cost, global access, compliance, resistance and toxicity. There is therefore a pressing need for a functional cure for HBV, defined as a sustained loss of viraemia and surface antigen (HBsAg) following termination of therapy(2). The impetus for this has been galvanised by recent successes in the cure of hepatitis C (HCV) and the immunotherapy of cancer.

Rational for an immunotherapeutic approach

One of the barriers to cure is the capacity of HBV to persist as covalently closed circular (episomal) DNA (cccDNA) within the nucleus of infected hepatocytes. The other main obstacle to HBV clearance is the profound immune exhaustion and T cell depletion characteristic of persistent infection. This is thought to be driven by the combination of decades of high dose antigenic stimulation and the tolerogenic environment within the liver (Figure 1,2).

A number of exciting new approaches are under development targeting different stages of the HBV lifecycle(3-5). However, it is unlikely that future antiviral drugs will be able to clear every trace of cccDNA from the large burden of infected hepatocytes. Hence the induction

of a strong immune response, able to effectively contain residual infection, is a compelling goal. Spontaneous, sustained resolution is achieved in most adults following acute infection but HBV is not eliminated and is readily reactivated if such subjects are immunosuppressed(6, 7). This exemplifies the fact that HBV is amenable to immune control. Long-lasting T cell responses have been documented following resolution(7) but decades after infection, immune control can persist even in cases without detectable circulating HBV-specific responses(8), suggesting relevant T cells may be sequestered in the liver(9) or that humoral immunity may contribute(8). Importantly, spontaneous control of infection with HBsAg seroconversion can occur in a small subset of patients even after establishment of chronic hepatitis B (CHB). Immune control of HBV is further exemplified by the resolution of CHB in recipients of bone marrow transplants from donors with HBV immunity. Thus the concept behind an immunotherapeutic approach to CHB lies in re-directing the failed immune response to mimic the features characterising spontaneous resolution. The goal is not to completely eliminate HBV but to achieve a functional cure, whereby residual traces are kept under tight immune control.

Although the immune correlates of HBV control are not completely understood, a coordinated response harnessing several synergistic aspects of immunity is likely to be an optimal therapeutic goal. This review will focus on the use of therapeutic vaccination and allied approaches to boost endogenous antiviral T cells, a critical arm of immunity to non-cytopathic viruses like HBV. However other immunomodulatory approaches in development may bypass the need for T cells or boost them indirectly by reducing viral load(10); the induction of B cell immunity should be an additional key objective(11).

In order to develop a rational immunotherapeutic approach, it is vital to first delineate the limitations of the immune responses present in patients with persistent infection. Another challenge is to decipher whether it is possible to disassociate responses mediating antiviral control from those driving immunopathology. ***Here we briefly review constraints on the T cell response in HBV and discuss how this knowledge could be applied to optimise the development of the future generation of therapeutic vaccines in combination with tailored immunotherapy.***

The HBV-infected liver: A multipronged attack on T cell immunity

Recent studies have provided unprecedented insights into the molecular mechanisms underlying the failure of T cell control in persistent viral infections (12-14). HBV-specific T cell responses circulate at modest frequencies even in acute resolving infection; they are further diminished in chronic infection (less than 0.2% of CD8(11)) and those remaining have profound functional defects. This attrition of functional antiviral responses is imposed by a combination of negative regulatory pathways together with prolonged high dose antigenic stimulation (Figure 1,2). The liver, receiving most of its blood supply from the portal vein, rich in gut antigens, is well-recognised to have a unique cellular composition that maintains tolerance in the healthy state (Figure 1 and reviewed(15)). Infection with HBV results in the immune system being bombarded by exceptionally high levels of virions and sub-viral particles for decades. In addition to repetitive TCR triggering by processed antigen driving T cell exhaustion(16, 17), it has been postulated that the large quantities of circulating soluble viral antigens (HBsAg, HBeAg) directly sabotage immunity. For example in mice, exposure to maternal HBeAg skews neonatal macrophage function to inhibit T cells(18).

HBV-specific T cells express high levels of the pro-apoptotic mediator Bim(19), a feature of T cells primed in the liver rather than the lymph node(20). In addition to their susceptibility to Bim-mediated apoptosis, HBV-specific T cells are vulnerable to deletion by the large fraction of activated NK cells within the liver(21). Such regulation of virus-specific T cells by NK cells has been shown to be a powerful rheostat controlling viral immunopathogenesis in murine models(22, 23). In the HBV-infected liver, CD4 T cells upregulate stress ligands, allowing them to bind to the high frequency of NKG2D-expressing NK cells and drive their activation and cytotoxicity(24). Liver-resident NK cells upregulate the death ligand TNF-related apoptosis-inducing ligand (TRAIL) in HBV infection(25, 26) and kill HBV-specific T cells, that selectively express the death receptor TRAIL-R2(21) (Figure 2).

Residual HBV-specific T cells that are not deleted are poorly functional upon cognate peptide stimulation, with markedly reduced production of the antiviral cytokine IFNγ, compared to T cells specific for well-controlled viruses within the same patients(27). This exhausted state is driven by multiple layers of co-inhibitory signals that outweigh positive co-stimulation(28). Not only do peripheral, and particularly intrahepatic, HBV-specific CD8 T cells express high levels of PD-1(29, 30), they also upregulate the other co-inhibitors like CTLA-4(31), Tim-3(32) and CD244 (2B4)(33). Hepatocytes and non-parenchymal cells such as Kupffer cells express PD-L1 and intrahepatic levels of this, and other co-inhibitory ligands such as galectin-9(32), increase further in the setting of viral hepatitis(9, 34).

IL-10 and TGFβ are prototypic immunoregulatory cytokines produced by many cell types in the tolerogenic liver environment that can further down-regulate T cell immunity(15). IL-10 induction tightly parallels increases in HBV viraemia in acute and chronic flares of disease(35, 36), with potential sources being the expanded populations of regulatory B cells and Tregs present in this disease(35, 37). IL-10 has been shown to be a key determinant of viral infection resolution versus persistence in mice(38, 39). It is classically regarded as an immunsuppressive cytokine that helps to dampen down tissue inflammation(40). However in HBV animal models, effector CD8 T cell production of IL-10 rescued HBV-specific CD8 T cells from apoptosis and enhanced acute liver pathology(41). Thus IL-10 exemplifies the highly context-dependent manner in which many coreceptors and cytokines regulate the balance between immunity and immunopathology.

Metabolic, mitochondrial and epigenetic defects in T cells

Recently we described a novel immunoregulatory mechanism characterising the HBV-infected liver. Granulocytic myeloid-derived suppressor cells (gMDSC) accumulate in patients with HBV infection, particularly those without liver inflammation and are further expanded within the intrahepatic compartment, where they have a more immunosuppressive phenotype. Amongst many potential regulatory mechanisms, we showed that gMDSC in patients with CHB had high expression and degranulation of the enzyme arginase I that catabolises the amino acid L-arginine, required for T cell proliferation(42). Our data suggested that starving T cells of nutrients like L-arginine in the

HBV-infected liver could downregulate immunopathology by suppressing bystander responses, whilst also limiting antiviral responses by HBV-specific T cells.

These data contribute to an emerging understanding that metabolic factors constitute an additional layer of checkpoints on T cell function, since mounting an effective response necessitates a rapid increase in biosynthetic and energetic capacity. We have found that HBV-specific T cells are unable to supplement glycolytic metabolism by switching to the more energy-efficient pathway of oxidative phosphorylation, likely attributable to an underlying defect in their mitochondria(43). This was evidenced by increased mitochondrial depolarisation accompanied by a paradoxical increase in mitochondrial mass. Importantly, an extensive analysis of HBV-specific T cells using unbiased transcriptomic analysis also identified fundamental defects centred on mitochondrial oxidative phosphorylation. Impaired mitochondrial polarisation and decreases in electron transport chain proteins were linked to the increased levels of baseline reactive oxygen species in HBV-specific T cells(44). Studies from other settings have simultaneously uncovered defects in mitochondrial biogenesis in PD-1^{hi} T cells in murine viral(45) and tumour(46) models. Consistent with the concept that fundamental cell-intrinsic abnormalities underpin the exhausted PD-1^{hi} phenotype, extensive epigenetic changes have also been described(47, 48). Taken together, these findings suggest potential new directions for future immunotherapeutic boosting of antiviral T cells, as discussed below.

The gold standard response: intrahepatic immunity in natural resolvers of HBV

Since the goal of functional cure in HBV treatment is to mimic the state of tight immune control achieved following natural resolution of infection, immune responses in the liver of such individuals could be regarded as the “gold standard”. We observed that CD8 T cells from the liver of a donor who had resolved HBV mounted immediate responses upon re-challenge with HBV peptides from all the major HBV proteins, producing the antiviral cytokine IFN-γ within 4 hours. The majority of these responses had the phenotype of tissue-resident memory T cells (T_{RM}, $CD69^{+}CD103^{+}$ or $CD69^{+}CD103^{-}$) congruent with their long-term persistence within the organ, poised to mount rapid antiviral function. T_{RM} provide vital roles in frontline immunosurveillance within many organs(49). Hepatic T_{RM} had distinct

features such as high IL-2 production(42) which can overcome PD-1-induced tolerance in the liver(50). More comprehensive study of this intrahepatic population is required to define other adaptations that may allow them to survive and function within the tolerogenic liver environment, providing a blueprint for the responses that immunotherapeutic approaches should aim to induce in HBV. We showed that it might be possible to promote induction of liver residence by sequential exposure of T cells to IL-15 and TGFβ, since this two-stpe cytokine signal can confer transcriptional and phenotypic features of liver-residency(9). Since T_{RM} are unable to recirculate it will be important to sample the liver as well as the blood in early-phase studies of novel immunotherapeutic agents for HBV.

The state of play with therapeutic vaccination for HBV

The goal of a therapeutic vaccine is to prime new antiviral responses and/or boost existing ones that are able to home to the liver and control HBV (Figure 3). To date there have been a number of therapeutic vaccines tested for HBV, as recently reviewed(51, 52); Table I lists examples of those currently in trial, along with alternative immunotherapies. Despite promising immunogenicity in pre-clinical studies, usually in uninfected mice, none have shown adequate efficacy when tested in patients with CHB. Here we consider whether it is possible to enhance therapeutic vaccine efficacy, looking beyond the use of classical adjuvants to consider specific manipulation of targeted T cells.

Bearing in mind what has been learnt from past failures and from our enhanced understanding of the inherent immune deficits that have to be overcome, how should the next generation of vaccines be optimised? Although this review concentrates on how to boost T cells, humoral immunity may contribute to the control as well as prevention of HBV infection(6, 53); developing a vaccine that is capable of inducing anti-HBs antibodies, the hallmark of functional cure, in an important consideration. In this respect a protein-based prime, that should be optimal for antibody induction, has advantages(52, 54).

However, the use of virally vectored vaccines, such as adenoviral vectors, has proven to be highly immunogenic in other settings and is currently being tested in patients ((55), Table I). The limitation of pre-existing adenoviral immunity can be circumvented using replication-

defective chimpanzee adenoviral vectors, as recently described for HCV. T cell immunogenicity is considerably enhanced by an MVA boost(56). Athough this approach was highly immunogenic when tested as a preventative vaccine in uninfected adults, results in HCV infected patients were disappointing(57, 58). These lessons from HCV are instructive for HBV since they are based on another persistent virus replicating in the tolerogenic liver niche and a similar construct is under development for HBV. In the therapeutic setting, the only HCV-specific T cells that could be boosted were those against viral sequences that were mismatched between autologous virus and vaccine immunogen and these T cells were not cross-reactive with the patient's virus(57, 58). Deliberately using heterologous antigen in vaccines has been proposed as an approach to increase T cell priming(54, 59) but it will only be effective if it is able to induce T cells with cross-reactive potential.

Beyond the vaccine formulation, additional considerations include the choice of HBV antigens and delivery route. An ideal therapeutic vaccine for HBV should aim for broad immunogens with cross-genotypic coverage. Although HBV is not as renowned for viral escape mutations as HIV and HCV, it does utilise the error-prone reverse transcriptase; CD8 T cell escape mutations have been described in patients with CHB(60) and recent research suggests their frequency may have been underestimated(8, 61). To combat the emergence of escape mutations, a therapeutic vaccine should therefore aim to induce a multispecific and polyclonal T cell response. Whilst T cell responses directed against epitopes within all major proteins have been detected at low frequency in CHB, their relative immunodominance is influenced by HLA and viral genotype and possibly also by the inflammatory environment(11); this hierarchy will anyway not necessarily correlate with protective capacity(62). There are good arguments for including core, polymerase and envelope in a vaccine construct: envelope to induce anti-HBs antibodies; envelope and core because these are presented at high levels on hepatocytes and responses to these antigens associated with control)(63); and polymerase because of emerging data that these responses may be better preserved in CHB (perhaps due to lower levels of antigen presentation)(11, 64, 65). More work needs to be done to better characterise the repertoire of HBV epitopes (most of those described to date are HLA-A2 restricted(66)) and to see how the specificity of both CD4 and CD8 responses generated by existing vaccines correlates with outcome.

Instead of pre-selecting immunogens, an alternative proposed approach uses the patient's personalised antigen reservoir for "autovaccination"; maturation of monocytes allows them to cross-present autologous HBsAg to expand T cells(67). In terms of vaccine delivery, a number of routes are being tested including subcutaneous & intranasal as well as intramuscular, with modifications such as electroporation to increase DNA delivery(68). Recent progress with malaria sporozoite-based prophylactic vaccines delivered intravenously to maximise hepatic uptake and promote the expansion of resident memory T cells(69) raises the question of whether this could be applicable in the setting of a therapeutic vaccine for HBV.

Checkpoint inhibitors to boost therapeutic vaccine responses

It remains uncertain whether the profound T cell depletion and complex immune defects in CHB can be overcome with the use of a more immunogenic vaccine alone. Can the immune system be better harnessed by combining vaccination with tailored immunotherapeutic manipulation? In a mouse model of persistent viral infection, blocking inhibitory signals such as PD-1(70) or IL-10 was able to enhance T cell immunogenicity of therapeutic vaccines(71). The use of checkpoint inhibitors such as PD-1 has gained traction from their remarkable efficacy in a subset of patients with various cancers, including HCC(72). Support for its applicability in CHB came from persistent infection with woodchuck hepatitis virus (WHV), a closely related hepadnavirus(73). In woodchucks with WHV suppressed on antivirals, multi-dose DNA vaccination supplemented by PD-L1 blockade resulted in enhanced virus-specific T cell degranulation, sustained off-treatment responses and HBsAg seroconversion in two of three animals(73). Recently, a phase I study evaluated anti-PD-1 treatment (Nivolumab) with or without therapeutic vaccination in patients with HBeAg-negative CHB suppressed on antivirals. Despite the fact that patients only received a single dose of Nivolumab, early unpublished data revealed a significant decline in HBsAg compared to baseline, with one out of 22 patients undergoing HBsAg seroconversion (74). Addition of a yeast-based vaccine GS-4774 showed no advantage over PD-1 blockade alone, but a more immunogenic vaccine could still prove to be beneficial in this combination.

However, PD-1 is not the only checkpoint inhibitor to consider targeting in CHB since multiple co-inhibitory receptors drive T cell exhaustion, as described above. PD-1 blockade was a logical first choice since responses to blockade *in vitro* (at least for HBeAg-negative low level carriers) are better for PD-1 than other co-inhibitory receptors(75). Combining blockade of PD-1 and CTLA-4 or PD1 and Tim-3 does show some synergistic and non-redundant reconstitution of HBV-specific T cells in vitro, although addition of a second blockade sometimes paradoxically abrogates the benefit of the first(31, 32). Therefore, and bearing in mind their toxicity profiles, combinations of multiple checkpoint inhibitors may not have a low enough risk-benefit ratio in CHB. The same concern may apply to the strategy of combining blockade of an inhibitory receptor with enhanced costimulatory signals to T cells, exemplified by *in vitro* restoration of HBV-specific immunity with PD-1 and 41BB(76).

Rather than combining multiple co-inhibitors or co-stimulators, it would be preferable if biomarkers could predict responsiveness to allow personalised selections for individuals or disease phases. Rapid advances in dissecting checkpoint molecular targets(77) should help to formulate biomarkers and underpin their rationale selection. For example PD-1 blockade has recently been shown to depend on restoring signalling through CD28, allowing CD28 to be used to predict PD-1 blockade efficacy in cancer(78), in line with previous data from CHB suggesting that the T cell differentiation phenotype could predict response(75). Similarly, deeper insights into the diverse cellular targets of different co-regulators and the realisation that some of their benefits on T cells are indirect via accessory or regulatory cells(79) could also help to select agents depending on the prevalent inflammatory mileu.

Although checkpoint inhibitors clearly have the capacity to rescue clinically relevant T cell responses *in vitro* and *in vivo*, it is becoming evident that exhausted T cells are not completely “burnt-out” but can continue to provide some active immunosurveillance(81). This is congruent with T_{RM} in the HBV-infected liver, which expressed very high levels of PD-1 yet secreted antiviral cytokines within a few hours of stimulation(9). Moreover, genetic deletion of PD-1 renders murine T cells susceptible to terminal differentiation and senescence(82), implying that PD-1 may serve as a protective brake to allow long-term

persistence of T cells in chronic high-dose infections. This highlights a potential limitation of repetitive PD-1 blockade in the setting of ongoing antigenic stimulation.

Other candidates for immune boosting of vaccination

More detailed studies of T cell phenotype and transcriptional profiles have identified subsets of PD-1^{hi}Eomes^{hi}Tbet^{lo} CD8 T cells with extensive epigenetic changes that cannot be re-wired by PD-1 blockade and therefore constrain their capacity to respond(47). The identification of exhaustion-specific enhancer modules provides new targets for genome editing that may make it possible to enhance the restoration of antiviral T cells by modifying their epigenetic landscape directly(48).

A more immediately tractable target in exhausted PD-1^{hi} T cells is the mitochondrial defects described earlier. Fisicaro et al demonstrated that functional HBV-specific CD8 T cells could be expanded by the addition of the mitochondrial antioxidants Mitoquinone or Mito-Tempo, that were able to act as ROS scavengers *in vitro*. Mitoquinone has previously been given to HCV patients and found to be safe and hepato-protective(83), making it an attractive candidate as a vaccine adjuvant in CHB. We found that IL-12 can also enhance mitochondrial polarisation in HBV-specific CD8 T cells and allow them to supplement glycolysis with oxidative phosphorylation for more efficient bioenergetics(43). This provides a metabolic mechanism to underpin the previous observation that IL-12 can rescue the antiviral function of HBV-specific CD8 T cells in a T-bet-dependent manner(27, 84). IL-12 can be incorporated within therapeutic vaccine plasmids, and is already being tested in the Innovio construct (Table I). An additional metabolic checkpoint of potential relevance in the setting of HBV is L-arginine, since arginase I-producing gMDSC and hepatocytes can catabolise this amino acid required for clonal expansion of T cells(42, 85, 86). A recent elegant study showed that elevating the supply of L-arginine to T cells induced global metabolic changes, with enhanced memory formation and survival in vivo(87).

A major strategy already being tested for CHB is the activation of immunity by TLR agonists. Whilst these primarily activate innate immunity, they are expected to exert secondary

changes on T cells (for example, activation of antigen presenting cells). TLR agonists have initially been considered as single immunotherapeutic agents in CHB (Table I) but it would be of value to test them in combination with therapeutic vaccines. For example the TLR-8 agonist GS-9688 induces a strong IFNγ response by intrahepatic innate cells (MAITs and CD56bright NK cells(88)), induced via IL-12 and IL-18(88), implying that TLR-8 might also harness the immunotherapeutic benefits of IL-12(27). Although these preclinical studies have provided some insights on mechanisms of action, further work to dissect their impact on the complex immune regulatory network within an HBV-infected liver may inform how best to combine them with therapeutic vaccines. Another immune modulator already being tested in Phase I trials for CHB is an inactivated parapoxvirus particle preparation (AIC649, Table I), proposed to induce cytokines via TLR-9 engagement(89). TLR-9 agonism has a strong rationale for use in CHB based on a study in an HBV mouse model showing it can enhance DNA vaccination to achieve control of infection. The mechanism of action of TLR-9 in this model was shown to be the induction of intrahepatic clusters of myeloid cells that form a protective cocoon around CD8 T cells, permitting their expansion away from the immunosuppressive influences of the liver(90).

The innate arm of the immune system can have negative as well as positive influences on T cell responses in CHB. It might therefore be of value to consider blocking innate responses capable of down-regulating HBV-specific T cells, as an adjunct to therapeutic vaccination. An example is the capacity of NK cells to delete HBV-specific T cells and contribute to their attrition in the infected liver(21, 24). One of the pathways we defined for this was induction of the death receptor TRAIL-R2 on HBV-specific T cells when activated by their cognate antigen. Interruption of this pathogenic cross-talk, for example by a small molecule able to block TRAIL, should limit the deletion of HBV-specific T cells activated by vaccine antigen and thereby enhance immunogenicity. A further potential benefit of such an approach is that it may simultaneously dampen bystander liver damage and hepatic flares by blocking the capacity of NK cells to kill TRAIL-R2-expressing hepatocytes. A potential concern has been that TRAIL blockade could accelerate liver fibrosis but our recent work suggests that the capacity of TRAIL to kill primary human hepatic stellate cells is counteracted by its ability to constrain their apoptosis by engaging inhibitory receptors TRAIL-R3/4(91).

Patient selection and safety considerations

The selection of patients in different clinical phases of CHB for trials of therapeutic vaccination and/or immunotherapy requires a combined consideration of efficacy, acceptability and safety. There are precedents in several HBV models, as well as in patients with CHB, of restoration of functional antiviral T cells resulting in HBV control without significant liver damage(73, 92, 93). Conversely, it is well-recognised that some degree of hepatic flare can be an indication of effective immune reconstitution; trials are therefore currently being designed to keep transaminase flares within safe limits rather than seeking to avoid them all together. In this regard, it makes sense to exclude patients who have cirrhosis or advanced liver fibrosis since they have less reserve of hepatic function to withstand an immune-mediated flare. Nevertheless, such patients are now starting to be treated with PD-1 blockade for HCC and initial results are promising, with no increase in the usual rate of adverse events seen with this checkpoint inhibitor(72). In the absence of an untreatable complication like HCC, the safety threshold for immunotherapy is obviously much higher in a well-tolerated chronic infection like HBV. Early results of the first single-dose study of PD-1 blockade in patients with CHB without HCC did not show significant flares of liver damage(74). However PD-1 is highly expressed by global as well as HBV-specific T cells within the liver, so autoimmune disease remains a potential concern for repeated dosing and longer follow-up.

Selecting patients well-suppressed on antivirals should optimise safety of any immunotherapy by reducing the number of infected hepatocytes that could be targeted by the reconstituted immune response and reducing any pre-existing liver inflammation(94). Furthermore, antiviral therapy may promote T cell boosting; studies in woodchucks showed enhanced antiviral responses to DNA prime/adenoviral boost in animals who had been pre-treated with Entacavir(93). In line with this, patients receiving antivirals also have some enhancement in the expansion potential of HBV-specific T cells, particularly in the first six months of treatment (95) or after several years of therapy(92). Patients with documented recent seroconversion to HBeAg (treatment-induced or spontaneous) may be a group particularly amenable to immune boosting, although evidence for this is lacking. Early results from treatment interruption trials suggest this strategy may further enhance antiviral

T cell recovery, so this is a setting that might be considered in the future, if a therapeutic vaccine had been found to be safe but insufficiently immunogenic in treated patients.

Nucleos(t)ide analogues suppress HBV DNA but do not reduce the burden of antigen load in CHB; alternative strategies to reduce viral antigens might therefore have additional beneficial effects on adaptive immunity. The rationale for this is supported by prime/boost vaccination of HBV transgenic mice showing an inverse correlation between induced T cell responses and pre-existing antigen levels(52, 54). The other current mainstay of treatment, PEG-IFNα, is able to reduce cccDNA and HBsAg levels but does not reconstitute HBV-specific T cells(96-98). In fact, results from patients with either HBV or HCV reveal that *in vivo* administration of IFNα tends to deplete global and virus-specific T cells and would not therefore be a helpful backbone upon which to base a therapeutic vaccine(96-99). However limited data suggest that combination or sequential nucleos(t)ide analogues and PEG-IFNα may be able to boost some T cell responses(100, 101)). Other molecules directly harnessing hepatocyte cell-intrinsic immunity to reduce cccDNA such as lymphotoxin-beta(102) or Rig-I/NOD-2 activator SB9200(103, 104)(Table I) may not have the downside of negative influences on T cells. New strategies aiming to lower HBV antigens, for example by RNAi(105, 106) should also reduce the amount of peptide/MHC presented to HBV-specific T cells. Other approaches such as nucleic acid polymers(107), inhibit secretion rather than production of HBsAg; any mechanisms by which they might aid with T cell reconstitution remain unspecified. Future trials of therapeutic vaccination should preferentially select patients who have optimal viraemia and viral antigen suppression with the latest combinations available to achieve this.

Another patient group that should be relatively safe and effective for testing immunotherapeutic strategies is those with well-established HBeAg-negative chronic infection. Selecting patients with very low levels of HBV DNA and HBsAg, repeatedly normal liver function and no evidence of liver fibrosis, who tend to have slightly stronger HBV-specific T cell responses(11) could maximise the chance that immune modulation will result in HBsAg seroconversion. On the other hand, an argument can also be made for targeting patients much earlier in the disease course, who would tend to be younger and may have less of marked an imprint of chronic infection and exhaustion on their T cell repertoire(108,

109). Moves to re-consider such immune-tolerant (HBeAg-positive chronic infection) patients for suppressive antiviral therapy in order to minimise the risk of DNA integration and carcinogenesis(110) could pave the way towards therapeutic vaccination and immunotherapy trials in this large, and highly infectious, patient group.

Conclusions

The therapy of HBV has entered a dynamic era, with many new options becoming available, to be tested alone and in combination. Rational trial design requires a detailed understanding of the underlying virological and immunological complexities of this infection. There will be opportunities to combine more immunogenic therapeutic vaccines with selective optimisation of responding T cells; enhanced knowledge of mechanisms constraining T cells should allow the definition of more precise targets for small molecule modulation. Here we have only considered therapeutic boosting of endogenous HBV-specific T cells. However an alternative compelling approach, particularly for HBV-related HCC, is the use of genetically engineered T cells, as reviewed elsewhere(111). Genetic introduction of HBV specificity using TCRs or chimeric antigen receptors bypasses the need to be able to recover the exhausted endogenous response, although such cells would still remain susceptible to these same constraints once they reached the infected liver. Therefore many of the immunological manipulations discussed here are also of potential relevance to the optimisation of genetically engineered T cells.

Much remains to be learnt about how to maximise the safety of immunotherapy in the inflamed HBV-infected liver. For example, delineating the extent of HBV hepatocytes containing HBV antigens and presenting HBV peptides within an infected liver in different disease phases and on antivirals would be very useful to determine the risk of T cell-mediated liver damage and the likely amount of “liver reserve”. Similarly, more studies of local immune responses compartmentalised within the liver are vital. New insights into the immune mechanisms underpinning hepatic flares and chronic necro-inflammatory damage will allow development of adjunctive approaches to limit any collateral damage induced by immunotherapy. Although toxic effects must be minimised, there will always be a delicate trade-off between immunity and immunopathology. Ultimately the hope is that a

combination of powerful direct-acting antivirals to minimise viral and antigen burden with the addition of tailored boosting of specific local immunity will allow safe, lifelong resolution of this infection.

Author Contributions

MKM wrote the manuscript. LJP carried out a literature and company search to construct the table of current trials, designed and made the figures and reviewed the manuscript.

Conflicts of interest

MKM's laboratory has collaborative grant funding from Gilead and Roche; MKM participates in advisory boards/provides consultancy to Gilead, Roche, Arbutus Biopharma, Immunocore.

LJP has participated in a Gilead advisory board.

Figure legends

Figure 1: Schematic of the human liver microenvironment with examples of liver-infiltrating and liver-resident cell subsets.
Myeloid-derived suppressor cell (MDSC), mucosal-associated invariant T cell (MAIT), liver-sinusoidal endothelial cell (LSEC)

Figure 2: Schematic depicting the features of T cell intrinsic and T cell extrinsic defects characteristic of T cells in the context of chronic viral infection.

Figure 3: Immunotherapeutic augmentation of therapeutic vaccination in CHB aims to expand a population of functional T cells, able to clonally expand and produce antiviral cytokines.

Table 1: Examples of novel candidate immune modulators and therapeutic vaccine currently in development/human clinical trial for the treatment of chronic HBV infection
*updated 07/2017

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